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BRIEF REPORT

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Decreased airway epithelial ion transport was associated with the severity of the respiratory syncytial virus infection and complications in infants

The respiratory syncytial virus (RSV) primarily infects airway epithelial cells. It also decreases airway epithelial sodium transport, especially through the epithelial sodium channel (ENaC),¹ which is crucial for osmosis-based fluid absorption across respiratory epithelium. Meanwhile, the electrochemical balance is maintained by secretion of chloride ions through apical chloride channels, including the cystic fibrosis transmembrane conductance regulator (CFTR).¹ In animals, ENaC inhibition has caused middle ear fluid collection in acute otitis media (AOM).²

We studied infants with RSV bronchiolitis, by analysing the messenger ribonucleic acid (mRNA) levels of the alpha, beta and gamma ENaC subunits and of CFTR in nasal epithelium. We hypothesised that the airway epithelial mRNA expressions of ENaC subunits would be negatively associated with the severity of RSV bronchiolitis, while CFTR would be positively associated. We also hypothesised that AOM would be associated with lower levels of ENaC mRNA and higher levels of CFTR.

Between January 2015 and February 2016, we recruited 30 children treated for RSV bronchiolitis at the Children's Hospital, Helsinki University Hospital, Helsinki, or at Turku University Hospital, Turku, Finland. The Ethics Committees of the Helsinki University Hospital and the Hospital District of Southwest Finland approved the study, and the parents provided written and informed consent. We studied children aged 1-12 months, hospitalised following a clinical diagnosis of bronchiolitis and with RSV confirmed by an antigen detection test or polymerase chain reaction of a nasopharyngeal sample. The exclusion criteria were prematurity, regular medication, chromosomal abnormalities and congenital cardiac or pulmonary malformation.

We collected a nasal epithelial scrape sample within 12 hours of admission, as a surrogate for the epithelium of the distal respiratory tract, and then measured relative expressions of alpha, beta and gamma ENaC and CFTR mRNAs, as previously described.³ The mRNA levels were normalised against epithelial cell-specific cytokeratin 18 to increase specificity of the mRNA analysis. TaqMan predeveloped assays (Applied Biosystems) were used for CFTR. We omitted four samples with only traces of total RNA or contaminated with genomic deoxyribonucleic acid.

Respiratory syncytial virus severity was assessed by the number of symptomatic days before sample collection, grade of

breathing difficulties during the hospital stay, length of hospital stay and total duration of illness. The grade of breathing difficulties was assessed by the mean respiratory rate, measured an average of 3 ± 2 times during the sampling day, the lowest pulse oximeter oxygen saturation, the mean capillary partial pressure of carbon dioxide and the need for non-invasive ventilation support or supplementary oxygen. Any AOM diagnosis was based on daily pneumatic otoscopy.⁴

We compared categorical variables with the chi-square test and continuous variables with the Mann-Whitney *U* test. Correlations were examined with Spearman's test. The level of statistical significance was 0.05. Statistics were analysed with SPSS, version 22.0 (IBM Corp) and Prism 7.0a. (GraphPad Software).

The patients' clinical characteristics are summarised in Table 1. None required intensive care. The mRNA expressions of ENaC subunits or CFTR showed no significant correlation with age or gestational age.

A negative correlation emerged between the mean RR and alpha ENaC mRNA expression ($n = 26$, $r = -.41$, $P = .038$). However, other measures of RSV severity or grade of breathing difficulties showed no correlation with the mRNA expressions of ENaC subunits or CFTR ($P > .05$ for all). There were no differences in the mRNA expressions of ENaC subunits or CFTR based on whether or not patients needed non-invasive ventilation or supplementary oxygen, or intravenous or nasogastric infusions. The same was true for fever or no fever. P was $>.05$ for all (data not shown).

The negative correlation between the respiratory rate and alpha ENaC mRNA expression suggests that ENaC expression was linked to the severity of RSV bronchiolitis, as reported by in vitro and in vivo animal studies that demonstrated reduced airway epithelial sodium transport due to RSV.^{1,5}

The 11 patients diagnosed with AOM had lower alpha, beta and gamma ENaC mRNA expressions than those without AOM (Table 1), but CFTR mRNA expression was similar in both groups.

To our knowledge, this was the first study on humans showing attenuated ENaC subunit expressions in AOM. The findings were consistent with rat studies,² where the expression of all ENaC

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	All patients n = 30	AOM n = 11	No AOM n = 19	P value ^a
Clinical characteristics				
Age (months)	1.9 (1.1-4.0)	1.3 (0.6-5.4)	1.9 (1.4-3.8)	.45
Gestational weeks at birth	39 + 5 (38 + 1-41 + 1)	39 + 4 (37 + 4-41 + 2)	39 + 5 (38 + 6-41 + 1)	.58
Male	16 (53%)	5 (46%)	11 (58%)	.51
Hospital stay (days)	3 (2-4)	2 (2-4)	3 (2-4)	.25
Symptomatic days prior to sample collection	4 (4-6)	5 (4-6)	4 (3-6)	.96
Respiratory rate	41 (37-45)	39 (32-46)	42 (38-44)	.52
Supplementary oxygen need	13 (43%)	5 (46%)	8 (42%)	.86
Capillary pCO ₂ level	5.6 (5.2-6.4)	5.5 (5.2-6.8)	6.0 (5.1-6.3)	.83
HFNT or CPAP ^b	9 (30%)	2 (18%)	7 (37%)	.28
Fever ≥ 38°C	12 (40%)	7 (64%)	5 (26%)	.04
Intravenous or nasogastric infusions	14 (47%)	4 (36%)	10 (52%)	.39
mRNA levels normalised against cell-specific cytokeratin 18				
Alpha ENaC	1.75 (1.23-1.94)	1.34 (0.93-1.74)	1.89 (1.52-1.96)	.03
Beta ENaC	1.53 (1.03-2.14)	1.06 (0.24-1.36)	1.87 (1.34-2.25)	.003
Gamma ENaC	0.93 (0.72-1.88)	0.74 (0.31-1.05)	1.52 (0.82-2.21)	.02
CFTR	0.37 (0.23-0.63)	0.38 (0.13-0.94)	0.37 (0.25-0.51)	.69

Note: Data are presented as numbers (percentages) or medians (interquartile ranges).

Abbreviations: CPAP, continuous positive airway pressure, HFNT, high-flow nasal therapy, pCO₂, partial pressure of carbon dioxide.

^aComparisons between RSV patients with and without AOM by the Mann-Whitney U test or chi-square test.

^bOne patient with AOM had CPAP compared to without AOM.

subunits in middle ear epithelium was reduced after condition resembling AOM was induced. The inhibition of airway and middle ear epithelial ENaC expression due to RSV could thereby predispose RSV patients to AOM.

Certain limitations should be considered. The cohort size may have lacked sufficient statistical power to demonstrate weak associations between the analysed parameters, and there were no controls. The results cannot be generalised to AOM caused by pathogens other than RSV and RSV bronchiolitis needing intensive care with mechanical ventilation.

This study suggests that airway epithelial ENaC mRNA expression was lower in patients with more severe RSV bronchiolitis symptoms and AOM. Thus, inhibition of airway epithelial ion transport may play a role in the pathogenesis of RSV bronchiolitis, especially in those with AOM. The role of airway epithelial ion transport in both RSV bronchiolitis and AOM warrants further systematic investigation.

CONFLICT OF INTEREST


None.

TABLE 1 Clinical characteristics and ENaC subunits and CFTR mRNA levels of RSV patients with and without acute otitis media

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